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Direct identification of pathogens from positive blood cultures using matrix-assisted laser desorption-ionization time-of-flight mass spectrometry

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Abstract

In recent years, matrix-assisted laser desorption-ionization time-of-flight (MALDI-TOF) mass spectrometry (MS) has proved a rapid and reliable method for the identification of bacteria and yeasts that have already been isolated. The objective of this study was to evaluate this technology as a routine method for the identification of microorganisms directly from blood culture bottles (BCBs), before isolation, in a large collection of samples. For this purpose, 1000 positive BCBs containing 1085 microorganisms have been analysed by conventional phenotypic methods and by MALDI-TOF MS. Discrepancies have been resolved using molecular methods: the amplification and sequencing of the 16S rRNA gene or the Superoxide Dismutase gene (sodA) for streptococcal isolates. MALDI-TOF predicted a species- or genus-level identification of 81.4% of the analysed microorganisms. The analysis by episode yielded a complete identification of 814 out of 1000 analysed episodes (81.4%). MALDI-TOF identification is available for clinicians within hours of a working shift, as oppose to 18 h later when conventional identification methods are performed. Moreover, although further improvement of sample preparation for polymicrobial BCBs is required, the identification of more than one pathogen in the same BCB provides a valuable indication of unexpected pathogens when their presence may remain undetected in Gram staining. Implementation of MALDI-TOF identification directly from the BCB provides a rapid and reliable identification of the causal pathogen within hours.

A comparative study of clinical *Aeromonas dhakensis* and *Aeromonas hydrophila* isolates in southern Taiwan: *A. dhakensis* is more predominant and virulent

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Abstract

Aeromonas dhakensis, often phenotypically identified as *Aeromonas hydrophila*, is an important human pathogen. The present study aimed to compare the clinical and biological features of *A. dhakensis* and *A. hydrophila* isolates from human wounds. A total of 80 *Aeromonas* wound isolates collected between January 2004 and April 2011 were analysed. The species was identified by the DNA sequence matching of *rpoD* and *gyrB* (or *rpoB* if necessary). Most of the *Aeromonas* isolates were identified as *A. dhakensis* (37, 46.3%), and 13 (16.3%) as *A. hydrophila*. Both species alone can cause severe skin and soft-tissue infections. More *A. dhakensis* isolates were found in wounds exposed to environmental water (32.4% vs 0%, p 0.042). More biofilm formation was noted among *A. dhakensis* isolates (mean optical density at 570 nm, 1.23 $\pm$ 0.09 vs 0.78 $\pm$ 0.21, p 0.03). The MICs of ceftriaxone, imipenem and gentamicin for *A. dhakensis* isolates were higher ($p < 0.0001$, < 0.04, and < 0.01, respectively). The survival rates of *Caenorhabditis elegans* co-incubated with *A. dhakensis* from day 1 to day 3 were lower than those of worms infected with *A. hydrophila* in liquid toxicity assays (all p values < 0.01). Isolates of *A. dhakensis* exhibited more cytotoxicity, as measured by the released leucocyte lactate dehydrogenase levels in human normal skin fibroblast cell lines (29.6 $\pm$ 1.2% vs 20.6 $\pm$ 0.6%, $p < 0.0001$). The cytotoxin gene *ast* was primarily present in *A. hydrophila* isolates (100% vs 2.7%, $p < 0.0001$). In summary, *A. dhakensis* is the predominant species among *Aeromonas* wound isolates, and more virulent than *A. hydrophila*.

Extension of the *Legionella pneumophila* sequence-based typing scheme to include strains carrying a variant of the *N*-acylneuraminate cytidylyltransferase gene

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Abstract

Sequence-based typing (SBT) combined with monoclonal antibody subgrouping of *Legionella pneumophila* isolates is at present considered to be the reference standard during epidemiological investigation of Legionnaires' disease outbreaks. In some isolates of *L. pneumophila*, the seventh allele of the standard SBT scheme, *neuA*, is not amplified, because a homologue that is refractory to amplification with the standard *neuA* primers is present. Consequently, a complete seven-allele profile, and hence a sequence type, cannot be obtained. Subsequently, primers were designed to amplify both *neuA* and the homologue, but these yielded suboptimal sequencing results. In this study, novel primers specific for the *neuA* homologue were designed and internationally validated by members of the ESCMID Study Group for *Legionella* Infections at national and regional *Legionella* reference laboratories with a modified version of the online *L. pneumophila* sequence quality tool. To date, the addition of the *neuAh* target to the SBT protocol has allowed full typing data to be obtained for 108 isolates of 11 different serogroups, namely 1, 2, 3, 4, 5, 6, 7, 8, 10, 13, and 14, which could not previously be typed with the standard SBT *neuA* primers. Further studies are necessary to determine why it is still not possible to obtain either a *neuA* or a *neuAh* allele from three serogroup 11 isolates.